

AWARD NUMBER: W81XWH-16-1-0760

TITLE: The role of tissue-resident donor T cells in rejection of clinical face transplants

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REPORT DATE: October 2017

TYPE OF REPORT: Annual

**PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 521702-5012**

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REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
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1. REPORT DATE October 2017		2. REPORT TYPE Annual		3. DATES COVERED 9/30/2016 to 9/29/2017	
4. TITLE AND SUBTITLE The role of tissue-resident donor T cells in rejection of clinical face transplants				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-16-1-0760	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Bohdan Pomahac, MD E-Mail: bpomahac@bwh.harvard.edu				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Brigham and Women's Hospital 75 Francis St Boston MA 02115				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT Unlike solid organ transplants, face transplants have a unique immunological characteristic – the presence of skin, which contains approximately 1 million T cell/cm ² . A full face transplant is 600-700 cm ² in size and therefore, contains approximately 600-700 million donor T cells. The long-term persistence of donor T cells within facial allografts and the role of donor vs. recipient T cells in face transplant rejection are two critical issues that have never been studied. We plan to (i) quantify the number and diversity of donor vs. recipient T cells within face transplants over time using cutting edge high throughput TCR sequencing (HTS), (ii) identify pathogenic T cell clones implicated in rejection, (iii) identify if these pathogenic T cell clones are of donor vs. recipient, and (iv) determine if these pathogenic T cell clones are measurable in blood during episodes of rejection and could therefore serve as an early and personalized biomarker of rejection.					
15. SUBJECT TERMS Nothing listed					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	14	19b. TELEPHONE NUMBER (include area code)

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1. INTRODUCTION:

Unlike solid organ transplants, face transplants have a unique immunological characteristic – the presence of skin, which contains ~1 million T cells/cm².¹ A full face transplant is 600-700cm² in size² and therefore, contains ~600–700 million *donor* T cells. Although the role of T cells in rejection of face transplants is well established, the role of donor T cells in the rejection process is unexamined. The aims of this project are to test the central hypothesis that donor T cells contribute to VCA rejection, and that pathogenic T cells (both donor and recipient-derived) are detectable in blood during rejection to serve as personalized rejection biomarkers.

2. KEYWORDS:

Face transplants, T cells, Rejection

3. ACCOMPLISHMENTS:

What were the major goals of the project?

List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Task 1. Obtaining HRPO approval – estimated at month 3, actual percentage of completion 100%. Completed on May 24, 2017.

Task 2. Determining turnover of donor and recipient T cells within facial allografts following transplant. Estimated to start at months 3-5, current percentage of completion is 43%.

Task 3: Confirmation of the role of pathogenic T cell clones in graft damage. Estimated completion: months 6-7. Current status: 0%

Task 4: Detection of pathogenic T cell clones in blood. Estimated completion months 8-10. Current status: 0%

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

Under Task 2, we have run high throughput T cell receptor CDR3 sequencing (HTS) using the banked tissues from three face transplant patients. Donor tissues collected before transplant were used to establish the donor T cell repertoire. Recipient tissues collected before transplant were used to establish the recipient T cell repertoire. Following this, banked skin biopsies from these three patients' face transplants, which had been collected at serial time-points following transplant were analyzed by HTS (Figure 1). Individual T cell clones that showed expansion during rejection were identified. These expanded clones were then assigned as either donor or recipient origin by comparing the CDR3 sequences to the known donor and recipient T cell repertoires.

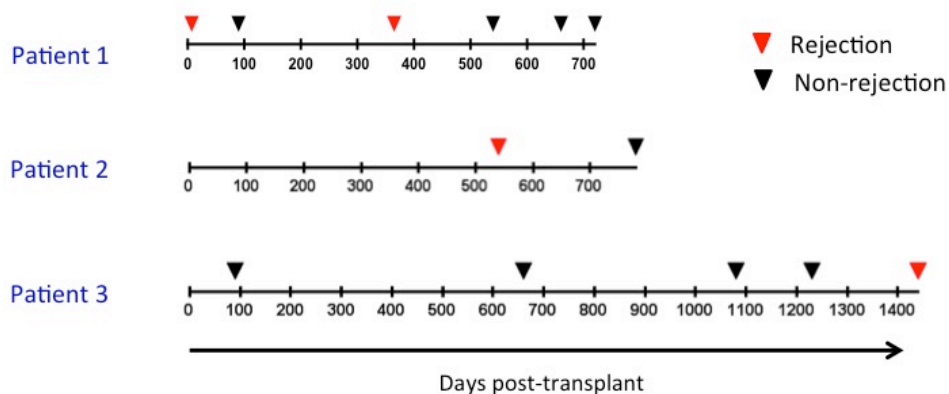
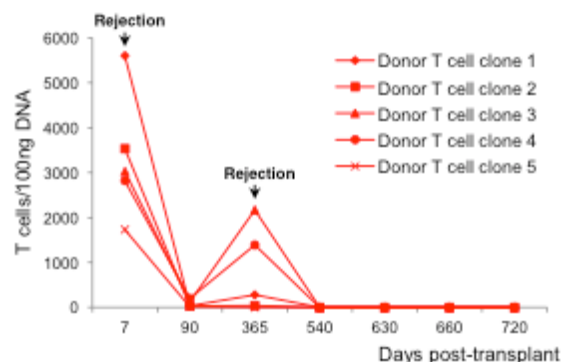


Figure 1. Timeline for skin biopsies analyzed by HTS.

Findings: Donor T cell clones predominated early within facial allografts, persisted for up to 1 year post-transplant and disappeared over time. Donor T cells showed clonal expansion during rejection, suggesting that they may participate in rejection (Figure 2). In contrast, the frequency of recipient T cell clones in the allograft increased progressively and dominated at later rejection episodes.

Figure 2. The total number of top 5 most abundant T cell clones in the skin of one face transplant (patient 1) showed that 5/5 clones that underwent clonal expansion during acute rejection episodes were donor-derived.



What opportunities for training and professional development has the project provided?

If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. “Training” activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. “Professional development” activities result in increased knowledge or skill in one’s area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Nothing to Report

How were the results disseminated to communities of interest?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to Report

What do you plan to do during the next reporting period to accomplish the goals?

If this is the final report, state “Nothing to Report.”

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

We plan to continue with Task 2: Quantification of donor and recipient T cells within facial allografts following transplantation. Towards this task, we are performing HTS of tissues collected prior to transplantation from the remaining face transplant recipients and donors to establish the donor and recipient T cell repertoires. Next, we will analyze banked skin biopsies from face transplants collected at rejection and non-rejection time points using HTS to identify the T cell clones that show clonal expansion. The total number and relative frequency of donor and recipient T cell clones in these skin biopsies will be determined by comparing the CDR3 sequences with the known donor and recipient T cell repertoire.

Over the next year, we plan to make progress towards Task 3: Confirmation of the role of pathogenic T cell clones in graft damage. We plan to use single nucleus RNA-sequencing to confirm the pathogenicity of clonally expanded donor and recipient T cells to determine their contribution to rejection.

In addition, we plan to make progress towards Task 4: Detection of pathogenic T cell clones in blood. Towards this task, we plan to perform HTS of peripheral blood mononuclear cells (PBMC) collected at the same time points as the biopsies we plan to utilize in Task 2, in order to determine if the same T cell clones that show clonal expansion within face transplants during rejection are detectable in peripheral circulation.

4. **IMPACT:** Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

What was the impact on the development of the principal discipline(s) of the project?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Nothing to Report

What was the impact on other disciplines?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to Report

What was the impact on technology transfer?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to Report

What was the impact on society beyond science and technology?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to Report

5. **CHANGES/PROBLEMS:** The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

Changes in approach and reasons for change

Describe any changes in approach during the reporting period and reasons for these changes.

Remember that significant changes in objectives and scope require prior approval of the agency.

There is no change in the objectives and scope of the project. We plan to change the approach for Task 3: Confirmation of the role of pathogenic T cell clones in graft damage. Instead of the method proposed initially (multiplex immunofluorescence staining), we plan to use cutting-edge single nucleus RNA sequencing (which only recently became available) of the banked skin biopsies from face transplant patients during rejection which will allow simultaneous determination of TCR sequences and unbiased analysis of their functional states. This will allow us to determine the pathogenicity of the expanded T cell clones.

Actual or anticipated problems or delays and actions or plans to resolve them

Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

HRPO approval took a little longer than we anticipated. It was approved on Month 7. However, we are now proceeding at full speed with the planned experiments.

Changes that had a significant impact on expenditures

Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to Report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Nothing to Report

Significant changes in use of biohazards and/or select agents

Nothing to Report

6. PRODUCTS: List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state “Nothing to Report.”

- **Publications, conference papers, and presentations**

Report only the major publication(s) resulting from the work under this award.

Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume; year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to Report

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: Author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to Report

publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk () if presentation produced a manuscript.*

Nothing to Report

- **Website(s) or other Internet site(s)**

List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to Report

- **Technologies or techniques**

Identify technologies or techniques that resulted from the research activities. In addition to a description of the technologies or techniques, describe how they will be shared.

Nothing to Report

- **Inventions, patent applications, and/or licenses**

Identify inventions, patent applications with date, and/or licenses that have resulted from the research. State whether an application is provisional or non-provisional and indicate the application number. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to Report

- **Other Products**

Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment, and/or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

- *data or databases;*
- *biospecimen collections;*
- *audio or video products;*
- *software;*
- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate “no change.”

Example:

Name: Mary Smith
Project Role: Graduate Student
Researcher Identifier (e.g. ORCID ID): 1234567
Nearest person month worked: 5

Contribution to Project: Ms. Smith has performed work in the area of combined error-control and constrained coding.
Funding Support: The Ford Foundation (Complete only if the funding support is provided from other than this award).

Name:	Bohdan Pomahac
Project Role:	PI
Nearest person month worked:	1
Contribution to Project:	Dr. Pomahac is a renowned surgeon-scientist. He provided scientific oversight and provided research samples utilized in the project.
Name:	Rachael Clark
Project Role:	Co-investigator
Nearest person month worked:	1
Contribution to Project:	Dr. Clark is a renowned skin immunologist and provided scientific oversight for the project.
Name:	Thet Su Win
Project Role:	Research Fellow
Nearest person month worked:	4
Contribution to Project:	Dr. Win has worked on regulatory submissions as well as the experimental procedures and data analysis.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Nothing to Report

What other organizations were involved as partners?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:

Organization Name:

Location of Organization: (if foreign location list country)

Partner’s contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner’s facilities for project activities);*
- *Collaboration (e.g., partner’s staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner’s staff use each other’s facilities, work at each other’s site); and*
- *Other.*

Organization Name: Broad Institute

Location of Organization: Cambridge, MA

Partner’s contribution to the project: Facilities, and “other”, where other means facilitating our performance of single nuclei RNA sequencing.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: N/A

QUAD CHARTS:

9. APPENDICES: N/A

The role of tissue-resident donor T cells in rejection of clinical

face transplantsRT150073

W81XWH-16-1-0760

PI: Bohdan Pomahac, MD

Org: Brigham and Women's Hospital **Award Amount:** \$150,000



Study/Product Aim(s)

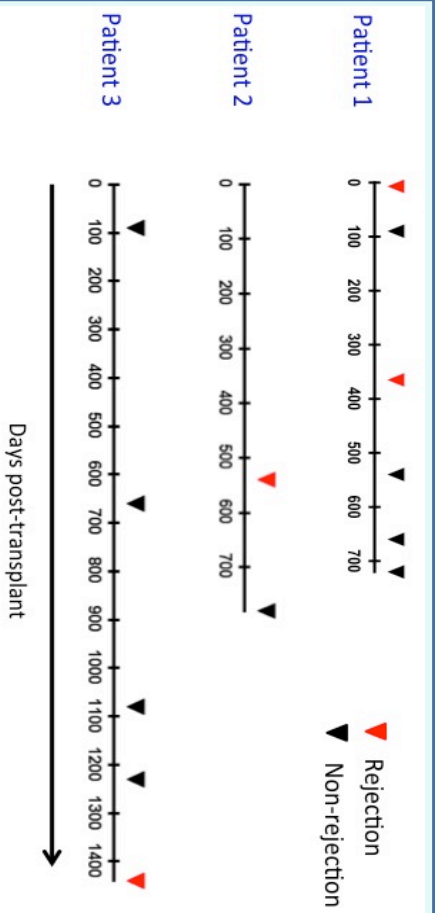
- Quantify the number and diversity of donor vs. recipient T cells within face transplants over time using cutting edge high throughput TCR sequencing (HTS)
- Identify pathogenic cell clones implicated in rejection using a combination of HTS and multicolor immunostaining/spectral imaging
- Identify if these pathogenic T cell clones are of donor vs. recipient origin
- Determine if these pathogenic cell clones are measurable in blood during episodes of rejection and could therefore serve as an early and specific biomarker of rejection

Approach

- Use skin biopsies collected from 6 recipients of face transplants at different time points.
- Correlate results with clinical findings.

Timeline and Cost

Activities	CY	15	16	17	18
Regulatory approval (Task 1)					
Quantity donor and recipient T cells (Task 2)					
Identify pathogenic T cell clones in grafts (Task 3)					
Identify pathogenic T cell clones in blood (Task 4)					
Estimated Budget (\$K)		\$0	\$37.5K	\$112.5K	\$0



Accomplishment: HRPO approval; analysis of three patient samples.

Goals/Milestones (Example)

CY16 Goals – Regulatory approval

☒ IRB submission and approval

☒ HRPO approval

CY17 Goal – Perform experiments, analyze and disseminate results.

☒ Quantify donor and recipient T cells in grafts

☐ Identify pathogenic T cell clones in grafts

☐ Identify pathogenic T cell clones in blood

☐ Disseminate results.

☐ **Comments/Challenges/Issues/Concerns**

• Modified timeline bars' length and positions.

Budget Expenditure to Date

Projected Expenditure: \$150,000

Actual Expenditure: \$88,231